HEPATIC REGENERATION FOLLOWING PARTIAL HEPATECTOMY IN THE CIRRHOTIC RAT MODEL AND THE EFFECTS OF EXOGENOUS PUTRESCINE ADMINISTRATION

A Thesis presented to the

University of Manitoba

In Partial Fulfillment of the Requirements for the Degree of Masters of Science in Surgery

by:

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MASTER OF SCIENCE

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ABSTRACT

There is conflicting data regarding the ability of the liver to regenerate following partial hepatectomy in animals and humans with cirrhosis. Hepatic regeneration is an essential component of the recovery period following partial hepatectomy. Unfortunately, tests which accurately will predict hepatic regenerative activity in the post-operative period have yet to be described. The present study was designed to document hepatic regeneration following partial hepatectomy in a carbon tetrachloride (CCl_4) model of cirrhosis and to determine whether exogenous putrescine, a polyamine that has been reported to stimulate hepatic regeneration and improve survival in animal models of acute liver failure, enhances hepatic regenerative activity in cirrhosis. In addition, hepatic collagen content was quantitated and an attempt made to determine if hepatic collagen content would correlate with and thus serve as a predictor of hepatic regenerative activity following partial hepatectomy in the rat CCl_4 liver injury model.

Hepatic fibrosis and cirrhosis were produced by weekly intragastric gavage with CCl_4 in 130 adult male rats. Vehicle gavaged rats (n=12) served as healthy controls. At surgery, 4 and 8 hours post 70% PHx, rats received either normal saline, 1 or 10 mg/kg of putrescine by i.p. injection. Additional CCl_4-treated rats were treated with putrescine (100 mg/kg) twice daily for 10 days prior to PHx as well as 0, 4 and 8 hours post PHx or normal saline. Hepatic regeneration was documented 24 and 48 hours post PHx by determination of restitution of liver mass, ornithine decarboxylase activity and [^{3}H]-thymidine incorporation into hepatic DNA. Hepatic collagen content was calculated at the time of partial hepatectomy by automated image analysis on Van Giesen stained liver tissue. This automated image analysis of the resected liver specimens further separated CCl_4-

treated rats into two subgroups; those with bridging fibrosis (fibrotic) and those with micronodular cirrhosis (cirrhotic).

Restitution of liver mass and ODC activity at 24 and 48 hours post PHx were similar in CCl_4-treated rats when compared to vehicle-treated healthy controls. Hepatic DNA synthesis, however, was significantly impaired at 48 hours in fibrotic rats (42.1 ± 20.6 DPM/mg DNA, $p<0.05$) and at 24 and 48 hours in cirrhotic rats (22.9 ± 9.6 and 27.0 ± 11.3 DPM/mg DNA respectively, $p<0.01$ and 0.005) when compared to vehicle-treated healthy controls (52.6 ± 9.2 and 78.3 ± 6.9 DPM/mg DNA at 24 and 48 hours respectively). Putrescine therapy prior to and/or following PHx did not alter restituted liver mass, ODC activity or DNA synthesis at 24 or 48 hours post PHx in fibrotic or cirrhotic rats when compared to their respective saline-treated controls. Although a significant inverse correlation was found between hepatic collagen content and DNA synthesis when all rats were considered (CCl_4- and vehicle-treated) at 24 and 48 hours post PHx ($r = -.4943$ and $-.7396$ respectively) ($p<0.05$), no such correlation existed when CCl_4-treated rats were considered independently ($r = -0.3231$ and -0.0910 at 24 and 48 hours respectively).

The results of this study indicate that hepatic DNA synthesis is impaired in rats with fibrosis and cirrhosis following partial hepatectomy. In CCl_4 injured livers, preoperative quantitation of hepatic collagen by automated image analysis does not serve as a useful predictor of hepatic regenerative activity. In this model of chronic liver disease exogenous putrescine does not enhance hepatic regenerative activity.

Prometheus Bound

"And when thou has brought to an end long length of time thou shalt come back to light; and the winged hound of Zeus, I tell thee, a blood-red eagle, shall fiercely tear thy body, limb meal, into great strips, coming, a guest unbidden, the lifelong day, and shall sup off thy liver till it be gnawed to blackness. Of hardships such as these look not thou for any end..."

ACKNOWLEDGEMENTS

I would like to acknowledge my supervisor, Dr. G.Y. Minuk, for his encouragement and support both during my research year and in my life endeavours subsequent to this. I would like to thank Tony Gauthier for his always willing assistance and instruction in the laboratory. I am indebted to all the members of the Liver Diseases Unit and thank them for their friendship.

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LIST OF ABBREVIATIONS

Abbreviations

CCl_4	Carbon tetrachloride
CCl_3	Trichloromethyl
DMNA	Dimethynitrosamine
CI	Collagen index
ODC	Ornithine decarboxylase
DNA	Deoxyribonucleic acid
PHx	Partial hepatectomy
DFMO	Difluoromethylornithine

INTRODUCTION

A. Statement of the Problem

The normal mammalian liver has a remarkable capacity to regenerate following injury or partial hepatectomy. Following partial hepatectomy morbidity and mortality rates are dependent on the remnant liver's functional capacity and its ability to regenerate in the post-operative period. In patients with cirrhosis, liver failure is a common and potentially fatal complication of partial hepatectomy (1-4). Although the mechanism of liver failure in this setting remains unclear, impaired hepatic regeneration appears to play an important pathophysiologic role.

B. Scope of the Problem

Primary hepatic malignancies occur in 10-20% of all patients with cirrhotic livers (5). Moreover, 60-70% of patients with hepatocellular carcinoma, the most common tumor in males world wide, and 15-20% of patients with cholangiocarcinoma have accompanying cirrhosis (5,6). Surgical resection in these patients will prolong survival and affords a chance for cure provided operative risks are minimized. Impaired hepatic regenerative activity is a significant factor contributing to the high morbidity (50%) and mortality (20%) seen in cirrhotic patients following partial hepatectomy (1,7-9). If hepatic regeneration could be enhanced the post-operative risk of liver failure would be reduced in all patients undergoing hepatic resection. In addition, cirrhotic patients currently denied potentially curative surgery for hepatic tumors because of an unacceptably high risk of liver failure in the post-operative period could be offered surgery.

C. Hepatic Regeneration

Hepatic regeneration is the inaccurate term commonly employed to describe the compensatory hypertrophy and hyperplasia that occur in the liver in response to damage or loss of tissue (10). The first scientific observers to suggest the possibility of regeneration of the liver were Cruveilhier (1909) (11) and Andral (1907) (12). The majority of scientific work in the field of hepatic regeneration has focused on the response to partial hepatectomy.

In the experimental literature there are conflicting views on the ability of the cirrhotic liver to regenerate in response to partial hepatectomy. Islami et al and Rabinovici et al using the carbon tetrachloride model of cirrhosis found that rats with cirrhosis regenerated in a manner similar to normal rats following partial hepatectomy (13,14). Indeed, Islami et al theorized that massive partial hepatectomy might be an effective therapy for the treatment of human cirrhosis. However, Mann et al in dogs, and Cameron and Karunaratue in rats could not demonstrate significant hepatic regeneration in response to partial hepatectomy in animals with carbon tetrachloride-induced cirrhosis (15,16). The reason for these discrepancies is that previous investigators employed imprecise measures such as liver weight, histology and gross appearance in documenting hepatic regeneration making it difficult to interpret results accurately. In addition, the times at which they made their observations following partial hepatectomy in these animal models are not clearly stated. In the clinical literature it has been generally accepted that human cirrhotic livers do not regenerate following partial hepatic resection (17). However, more recently it has been shown that human cirrhotic livers do regenerate but that the rate of regeneration is lower than normal (18). It appears that complete restoration of the residual liver is possible in some patients with cirrhosis.

A pre-operative predictor of hepatic regenerative activity would aid in the identification of those patients with cirrhosis who could tolerate partial hepatectomy. Pre-operative evaluation of the cirrhotic patient considered for hepatic resection must include an estimate of hepatic functional reserve. There is a critical functional mass of liver which is necessary to sustain life. The accurate assessment of hepatic functional reserve is a difficult problem. Methods range from simple classification systems to more complex measures of hepatic function. The Childs classification (19) appears to be a reliable predictor of long term mortality and is used to exclude patients at exceedingly high risk from hepatic resection (1,2). However, a more sensitive measure of hepatic function is needed to predict early morbidity and mortality. Estimates of hepatic blood flow such as indocyanine green clearance are no more sensitive then Childs classification in predicting post-operative outcome in cirrhotic patients following partial hepatectomy (8). Promising predictors of operative mortality focus on the mitochondrial function of hepatocytes and include cytochrome a ($+a_3$) content and redox tolerance index (20,21). Any correlates of hepatic regenerative activity should accurately reflect remnant hepatic function in the post-operative period. These correlates would then serve as markers to predict which cirrhotic patients could tolerate partial hepatic resection.

Factors which control hepatic growth and the sequence of events which culminates in restitution of functional hepatic mass remain largely undefined. Recent investigations have focused on identifying the agents involved in the hepatic regenerative response to partial hepatectomy. Several stimulatory (polyamines, insulin, glucagon, endotoxin) and inhibitory (ammonia, mercaptans, GABA) compunds have been studied. The polyamines, particularly putrescine, are

essential for cell growth and have emerged as an important component of the hepatic regenerative response. The importance of putrescine as a liver growth promotor has resulted in the successful treatment of acute fulminant hepatic failure in humans with exogenous putrescine.

OBJECTIVES

1. To create the carbon tetrachloride (CCl_4) model of experimental cirrhosis in Sprague-Dawley rats.
2. To document the regenerative capacity of a cirrhotic liver in the CCl_4 rat model.
3. To document the degree of hepatic fibrosis using an estimate of collagen content in the rat CCl_4 model of cirrhosis.
4. To determine if degree of hepatic fibrosis correlates with hepatic regenerative activity following partial hepatectomy in rats.
5. To compare the rate of hepatic regeneration in putrescine versus saline treated cirrhotic rats following partial hepatectomy.
6. To document concentrations of putrescine in cirrhotic versus non cirrhotic livers following partial hepatectomy in rats.

THE MODEL

A. Models of Cirrhosis

Experimental production of cirrhosis has been accomplished in several animal species using a variety of techniques. However, the production of severe cirrhosis in suitable numbers of animals with any degree of consistency continues to be a problem regardless of the model employed.

i) Hepatotoxins

The most popular hepatotoxin used for experimental induction of hepatic fibrosis is carbon tetrachloride (CCl_4). CCl_4 in repetitive doses was shown to produce cirrhosis in rat livers in 1936 and has since been used in other animal species by a variety of routes with similar results (22,23). CCl_4 requires bioactivation by mixed function oxidases which yields the reactive metabolite, trichloromethyl radical (CCl_3). This free radical can initiate lipid peroxidation or react with sulfhydryl groups of proteins resulting in acute liver damage (24,25). The addition of phenobarbital in drinking water has been shown to potentiate the hepatotoxicity of CCl_4 through the induction of isozymes of cytochrome P450 (26,27). To produce experimental cirrhosis it is necessary to give repeated doses of CCl_4 with each resultant episode of liver damage confined within a narrow range between a reversible hepatitis on the one hand and death by acute liver failure on the other. Two problems have been repeatedly observed with this model: (a) the response of individual animals to CCl_4 is variable and (b) mortality during the first weeks of treatment is 30-60%. However, Procter and Chatamra have reported a 76% yield of cirrhosis using the CCl_4 model in Wistar rats achieved with 8-10 doses of CCl_4 (28).

Dimethynitrosamine (DMNA) and thioacetamide are other hepatotoxins which will produce hepatic necrosis and subsequent fibrosis in many animal species.

However, both agents when administered in chronic low doses produce hepatic tumors (29-31).

ii) Nutritional Models

Diets low in protein and choline with a high level of fat produce cirrhosis in rats (32,33). There are many disadvantages to this model including a variable response among animals, a prolonged time course to achieve cirrhosis and distinct species differences in the susceptibility to choline deficiency.

A diet low in protein and methionine supplemented with 0.5% DL-ethionine is fibrogenic in young rats (34). However, the high mortality and inconsistent induction of fibrosis and cirrhosis make this model unsuitable for long term experiments.

iii) Immunologic Models

Administration of certain substances can trigger immune-mediated cellular responses thereby inducing hepatic fibrosis. These antigenic substances have included heterologous serum, bacterial cell wall products, and endotoxin (35-38). It is not yet known whether the hepatic lesions seen in these models can be extrapolated to conditions associated with the more common types of human liver fibrosis.

iv) Biliary Cirrhosis

Obstruction of the common bile duct will produce cirrhosis in a number of animal species. However, the technical problems inherent to this model may negate its usefulness. If the obstructed ductal system recanalizes the histologic changes induced will revert to normal. There is a high mortality associated with this model both at the time of bile duct ligation and with

subsequent development of biliary obstruction. In addition, the interspecies differences in the response to biliary obstruction are noteworthy (39-42).

v) Alcohol

Alcohol constitutes a major etiologic factor in human liver fibrosis. This has generated considerable interest in developing animal models of this disease. However, alcohol in volumes that animals will voluntarily consume is not an adequate hepatotoxin for the production of cirrhosis within the lifespan of smaller laboratory animals (43). The Lieber-DeCarli liquid diet with ethanol as a part of a nutritionally defined diet has induced the development of fatty liver in rats (44). However, liver necrosis and fibrosis could not be demonstrated in rats using this model. The Tsukamoto-French rat model employs intragastric infusion of alcohol via a chronically implanted gastric catheter to achieve high alcohol intake in rats (24). This model has resulted in advanced hepatic lesions including necrosis and fibrosis over relatively short periods of time (12 weeks).

B. Choice of Model

An ideal model of liver fibrosis, ie one duplicating the morphologic features seen in human disease with subsequent development of pathophysiologic sequelae, has not been achieved with a high degree of reproducibility and low mortality. The model familiar to most investigators and reported to result in the highest yield of fibrosis/cirrhosis is the carbon tetrachloride model (28). Experimental CCl_4-induced cirrhosis produces a gradual and discrete progression of pathologic changes. The CCl_4 model has three major advantages over other models: 1) an acceptable period needed for the development of liver cirrhosis, 2) a relatively low mortality rate and 3) toxic administration can be individually regulated to obtain uniform liver damage in most of the treated animals. The CCl_4 model has been used with success in the experimental production of decompensated micronodular cirrhosis duplicating the morphologic and biochemical features seen in human liver cirrhosis. A major concern is that, if, at any time during CCl_4 treatment, the administration of CCl_4 is stopped too early, or becomes too little to sustain the pressure on the liver, the liver is likely to revert at least part way back towards normal (27). Therefore, it is likely that the most reliable and relatively stable point for comparative studies is when the process reaches the fully developed decompensated micronodular stage. However, the mortality among this group is considerable particularly if a further insult such as surgery is planned as part of the experimental protocol. Under these conditions, it is most useful to terminate the model at an earlier time point when the animals have compensated liver cirrhosis.

MATERIALS AND METHODS

A. Experimental Protocol

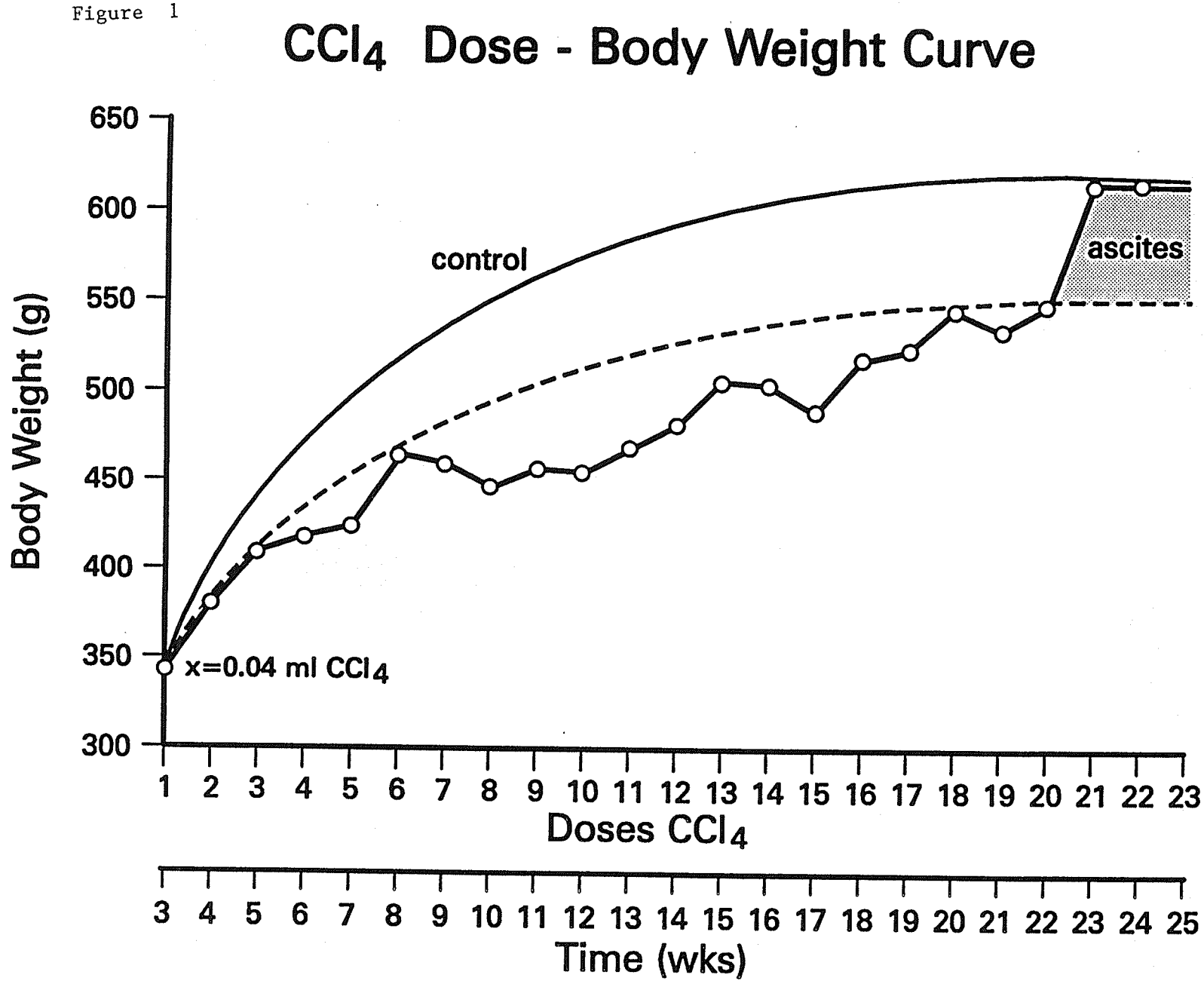
Animals: One hundred and sixty five male Sprague-Dawley rats weighing 125-175 grams were housed in cages with a 12 hour light and 12 hour dark cycle at 23°C. The diet for all animals was regular laboratory chow ad lib. All animals had free access to phenobarbital in tap water (concentration 35 mg/dl) as their only source of drinking water throughout the duration of the experiment. One hundred and fifty rats were chosen for CCl_4 administration while the 15 remaining rats were maintained as healthy controls.

Induction of Cirrhosis: After two weeks of phenobarbital for maximal induction of liver enzymes carbon tetrachloride administration was begun. CCl_4 in olive oil was administered on a once weekly dosing schedule to total 20-22 doses for the experimental rats. Following light ether anaesthesia CCl_4 was injected intragastrically using a gavage needle (4.0 inches). Healthy control rats received a similar volume of the vehicle by an identical route once weekly. The calibration dose of CCl_4 was 0.04 ml and chosen to minimize mortality. Subsequently, the weekly doses of CCl_4 were adjusted, up to a maximum of 1.20 ml, according to the body weight change caused by the previous dose (figure 1). Using this model all rats required unique dosages of CCl_4. The final dose of CCl_4 was give 5-6 days prior to surgery.

Surgery: Partial (70%) hepatectomy was performed on CCl_4-treated and healthy control rats anaesthetized with ether after a midline laparotomy by aseptic removal of the median and left lateral lobes according to the procedure of Higgins and Anderson (45). Once removed a biopsy of the left lateral lobe was taken and placed in 10% formalin for subsequent histologic examination.

Histopathology: Representative blocks of each resected liver were

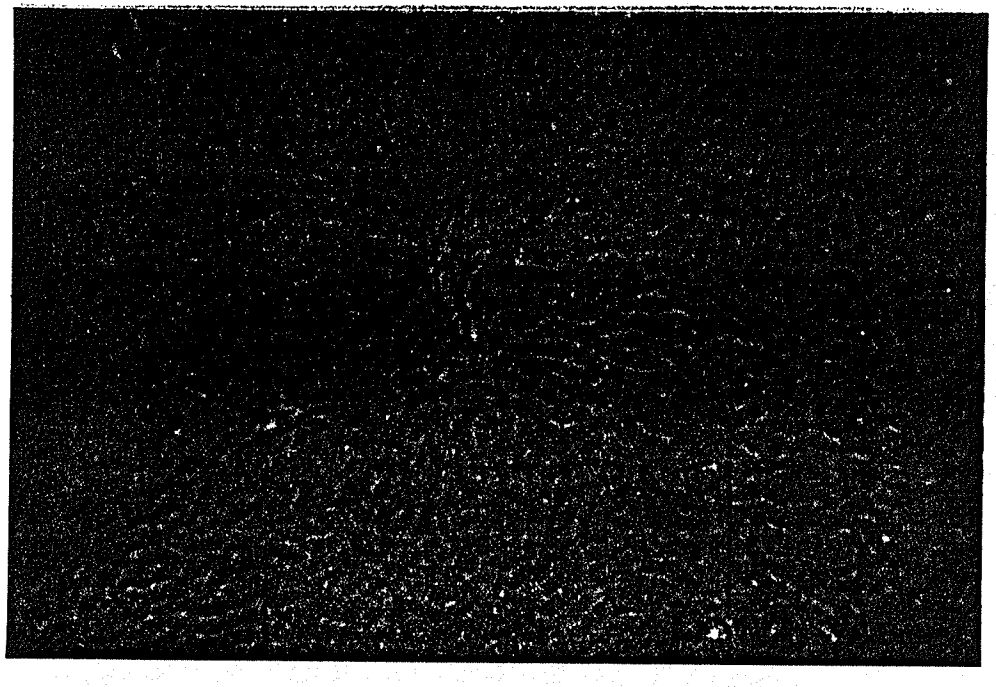
Figure 1


CCl4 Dose - Body Weight Curve
Body Weight (g)
650
600
550
500
450
400
350
300
control
ascites
x=0.04 ml CCl4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Doses CCl4
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Time (wks)

prepared. After routine parawax processing hematoxylin and eosin sections were obtained and the degree of liver damage assessed under light microscopy. Fibrosis and nodule formation were recorded on the coded blocks. In addition, mature collagen was differentially stained deep red by the Van Giesen method (a solution of 1% aqueous acid fuchsin, saturated aqueous picric acid and concentrated hydrochloric acid). The proportional area of collagen formation was determined using computer controlled image analysis (Bioquant Automated Meg 4 Program; R & M Biometrics, Inc., Nashville, Tennessee, USA). For each rat the area of stained collagen in four randomly selected fields was measured and the average expressed as the area of mature collagen per unit area of liver tissue; termed the collagen index. As a result of histologic analysis rats were further divided into three groups - healthy controls with a collagen index less than 5.0 units, CCl_4-treated rats with a collagen index 5.0-19.9 (fibrotic group), and CCl_4-treated rats with a collagen index greater than 20.0 units (cirrhotic group)(see photos 1-3).

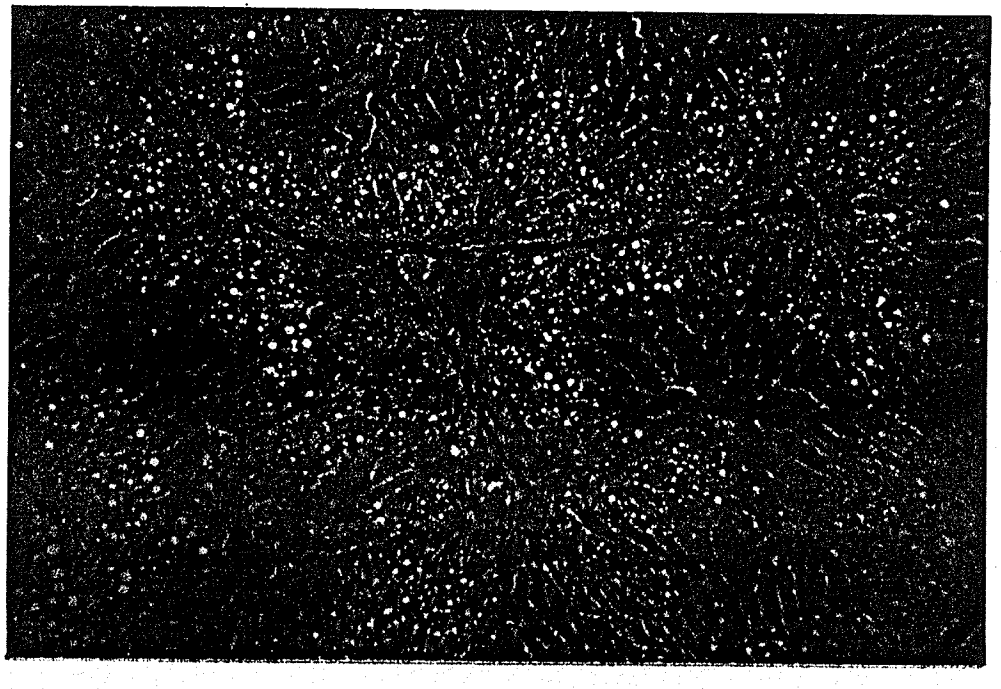
Putrescine Administration: At the time of surgery CCl_4-treated rats were randomly allocated to one of three treatment arms. One group received 1 mg/kg putrescine, the second group 10 mg/kg putrescine and the third group an identical volume of normal saline by intraperitoneal injection. Putrescine solutions were buffered to pH 7.4 with 1.0M NaOH. Putrescine or saline was administered at surgery, four and eight hours post-operatively. Vehicle-treated control rats received an equal volume of normal saline by intraperitoneal injection at the same time intervals as those described for CCl_4-treated rats. An additional group of 6 CCl_4-treated rats received 100 mg/kg of putrescine twice daily for 10 days prior to partial hepatectomy and again 0, 4 and 8 hours post

PHOTO 1



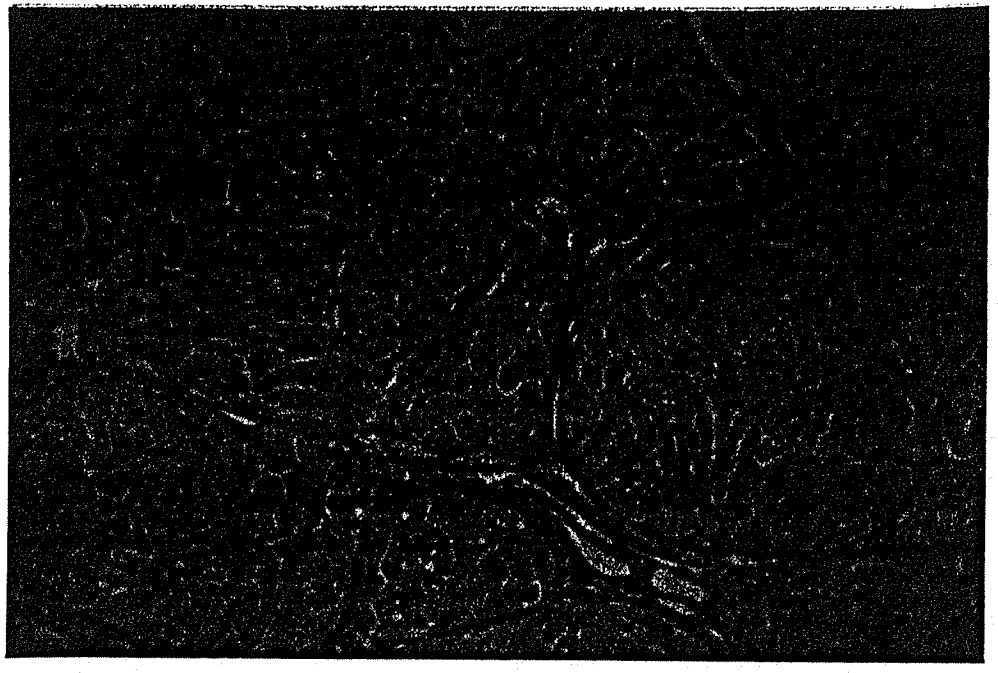
HEALTHY
CONTROL
C.I.=1.2

PHOTO 2



FIBROTIC
C.I.=13.4

PHOTO 3



CIRRHOTIC
C.I.=36.3

partial hepatectomy. These rats were sacrificed at 48 hours only. The control CCl_4-treated rats for this group (n=6) received normal saline in an identical fashion.

Liver Tissues: [^{3}H]-thymidine (10 uci/200 g body weight) was injected intraperitoneally one hour prior to sacrifice for determination of DNA synthesis. Rats were sacrificed at 24 or 48 hours post partial hepatectomy by exsanguination following ether anaesthesia. The remaining right lateral lobe and caudate lobe of the liver were rapidly excised by midline laparotomy, weighed and placed immediately on dry ice. Within weeks from surgery liver tissues were homogenized 1:9 (w/v) in 100 mM sodium phosphate buffer, pH 7.2 containing 5 mM dithiothreitol for subsequent tissue assays.

B. Parameters of Hepatic Regeneration

i) Percent Restitution of Liver Mass

The entire resected liver specimen was weighed at the time of partial hepatectomy and this weight was taken as 70% of the total prehepatectomy liver weight. Thus, the total prehepactectomy liver weight was calculated as (100/70) x resected liver weight. At the time of sacrifice the remaining liver was excised and weighed. The percent restitution of liver mass is the remnant liver weight divided by the calculated total prehepatectomy weight and multiplied by 100 to yield a percentage.

ii) ODC Activity

ODC activity was measured in the liver homogenates by the quantitation of $^{14}CO_2$ liberated from ^{14}C-labeled substrate, ornithine as described by Luk and Baylin (46). The reaction mixture contained 0.20 ml of liver homogenate, 0.05 ml of pyridoxal phosphate (4 mM) and 0.05 ml of 2.5 mM ^{14}C-ornithine (New England Nuclear, Boston, Mass.) in a total volume of 0.2 ml of buffer containing 5 mM Na H_2 PO_4, 5 mM Na_2 HPO_4, 0.1 mM EDTA and 2 mM dithiothreitol, pH 7.4. After a two hour incubation at 37°C the reaction was stopped with 0.5 mL of 50% trichloroacetic acid. The $^{14}CO_2$ liberated by the decarboxylation of ornithine was trapped on a piece of GF-C filter paper impregnated with 200 ul of hyamine hydroxide, which was suspended in a centre well above the reaction mixture. The $^{14}CO_2$ trapped in the filter paper was measured by liquid scintillation spectroscopy and ^{14}C-radioactivity expressed as counts per minute per microgram of protein. Protein contents of liver homogenates were assayed by the method of Hartree using bovine serum albumin as the standard (47).

iii) DNA Synthesis

DNA synthesis was estimated by [^3H]-thymidine incorporation

into DNA over the one hour period before death. A 0.25 ml aliquot of the 10% liver homogenate was precipitated with 50% trichloroacetic acid and then centrifuged at 3000 rpm for 15 minutes at 4°C. The pellet was then resuspended in tissue solubolizer, capped and incubated at 50°C for 48 hours. The [^{3}H]-radioactivity was determined in the washed pellets of liver homogenates in a Beckman LS 9800 liquid scintillation counter. DNA content of the pellets was measured by reaction with 3,5 diaminobenzoic acid at 37°C.

C. Tissue Polyamine Analysis

At the time of sacrifice, livers were excised and homogenized 1:9 (w/v) in a 100 mM sodium phosphate buffer containing 5 mM dithiothreitol. Homogenates were deproteinized by the addition of sulfa salicyclic acid in the presence of an internal standard (1,6-hexanediamine), centrifuged at 3000 g for 15 minutes and the supernatants adjusted to a pH of 2.2 with 0.4 mol/l NaOH before analysis. Levels of putrescine and its metabolites: spermidine and spermine were obtained from an Alpha Plus Amino Acid analyser (LKB Biochrome Ltd., Cambridge, U.K.) equipped with an 80 mm x 40 mm stainless steel column packed with ultropac 8 (8 $\pm$ 1 um) cation resin and an LKB 4460 fluorescence detector with orthophaldehyde as the fluorescence reagent. Amino acid elutions were carried out with buffer 1 (0.2 mol/L Na^+ citrate, pH 4.25) for 25 minutes at 60°C, followed by buffer 2 (1.2 mol/L Na^+ citrate, pH 6.45) for 10 minutes at 70°C, followed by buffer 3 (Na^+/K^+ citrate mixture containing 0.56 mol/L Na^+, and 1.6 mol/L K^+, pH 5.60) for 10 minutes at 70°C. Buffer 3 was continued for a further 20 minutes and the temperature was raised to 90°C. Regeneration and equilabration of the column was accomplished with buffer 4 (0.40 mol/L NaOH) for 5 minutes at 90°C, followed by buffer 1 for 15 minutes at 60°C. Buffer flow was 35 ml/hr and reagent flow was 17 ml/hr. Automatic integration was performed by a Nelson 900 series integrator (Nelson Analytical Inc., Cupertino, CA.) Tissue concentrations were expressed as picomoles per milligram of protein.

STATISTICAL ANALYSIS

The results provided represent the mean $\pm$ S.D. for five to nine rats per group. Statistical analysis was performed using one-way ANOVA, student's t test and regression analysis. Values of $p < 0.05$ were considered significant.

This study was approved by the University of Manitoba Animal Ethics Review Committee.

RESULTS

Histopathology

All vehicle-treated control rats had a collagen index less than 3.5 units and normal liver histology as evaluated by light microscopy. All CCl_4-treated rats exhibited varying degrees of hepatic injury and the collagen index was 5.0 or greater. Rats with histologic evidence of bridging fibrosis had a collagen index greater than five but less than 20.0. A collagen index of 20 or greater was consistent with light microscopic findings of micronodular cirrhosis.

Deaths

Following partial hepatectomy a total of nine deaths occured. One death (6%) occured in the control (saline-treated) fibrotic group and two deaths (12%) in the control cirrhotic group. No deaths occured in the 12 healthy control rats. In putrescine-treated fibrotic rats one (6%) and one (6%) deaths occured in rats who received 1 mg/kg or 10 mg/kg putrescine respectively. In putrescine-treated cirrhotic rats three (19%) and 1 (6%) death(s) occured in these two groups respectively. In rats treated with either saline or putrescine at the time of partial hepatectomy, three (19%) and 6 (38%) deaths occured respectively.

Hepatic Regeneration in Surviving Animals

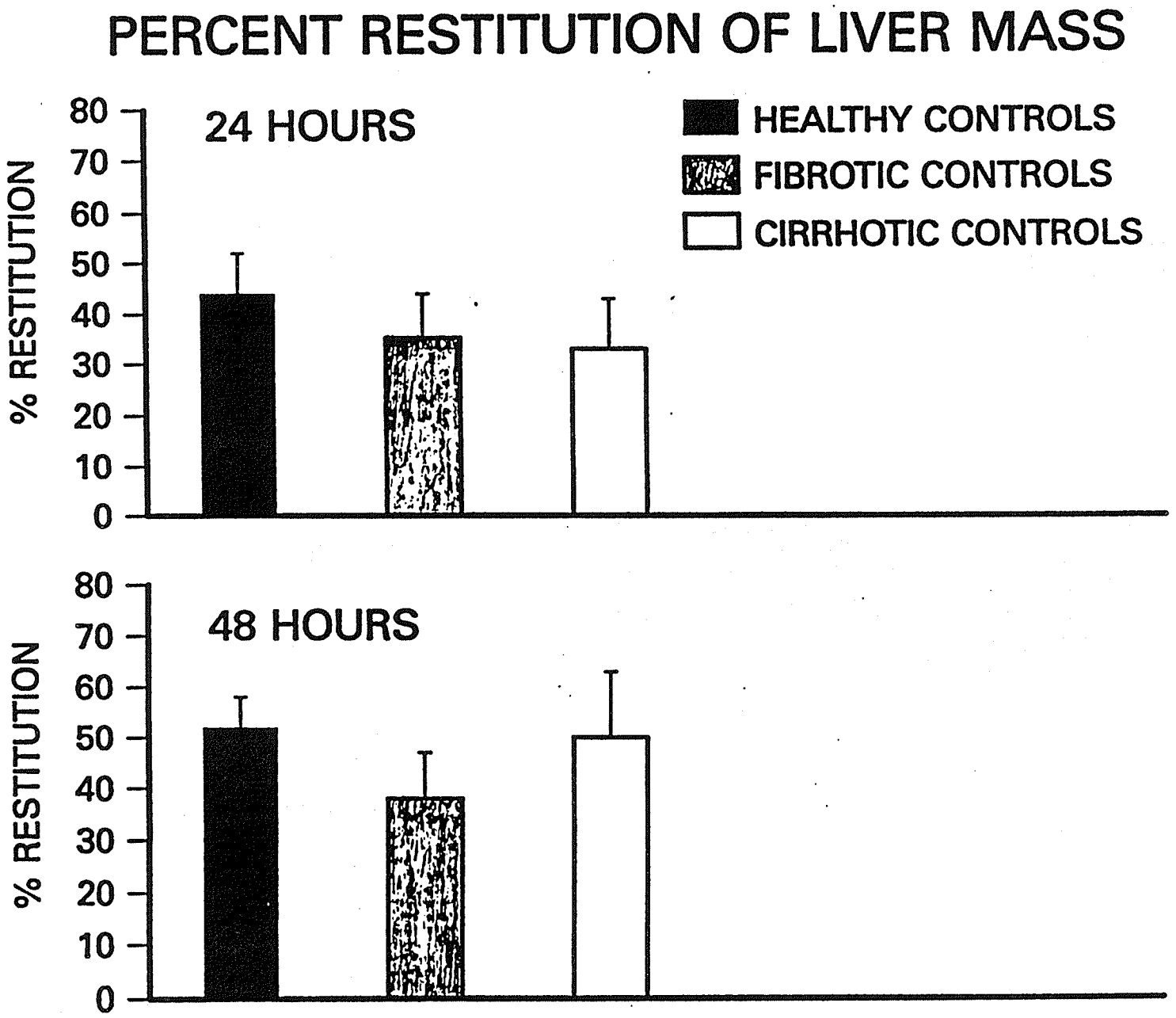
Restitution of Liver Mass:

Restitution of liver mass did not differ between vehicle-treated control rats and CCl_4-treated rats (fibrotic or cirrhotic) at 24 or 48 hours post PHx (figure 2).

ODC Activity

As with restitution of liver mass, no differences were observed between hepatic ODC activity in vehicle-treated control rats compared to CCl_4-

Figure 2


PERCENT RESTITUTION OF LIVER MASS
24 HOURS
48 HOURS
% RESTITUTION
% RESTITUTION
80
70
60
50
40
30
20
10
0
HEALTHY CONTROLS
FIBROTIC CONTROLS
CIRRHOTIC CONTROLS

treated rats (fibrotic or cirrhotic) at either 24 or 48 hours post PHx (figure 3).

DNA Synthesis

As shown in figure 4, DNA synthesis was significantly reduced in fibrotic rats at 48 hours post PHx when compared to vehicle-treated control rats ($p < 0.005$). In cirrhotic rats, DNA synthesis was reduced at both 24 and 48 hours post PHx compared with vehicle-treated control rats $p < 0.0005$).

Hepatic regenerative activity revealed a significant inverse correlation with the collagen index at 24 and 48 hours post PHx when all rats were considered (CCl_4-treated and controls). The correlation coefficient at 24 hours was $r = -0.4943$ and at 48 hours $r = -0.7396$ ($p < 0.05$) (figure 5). However, when only CCl_4-treated rats were considered, no correlation existed at 24 or 48 hours ($r = -0.3231$ and $r = -0.0910$ respectively). Moreover, when the range of normal hepatic regenerative activity was calculated in vehicle-treated controls, no specific collagen index could be identified to reliably predict normal or impaired regenerative activity following partial hepatectomy.

Hepatic Putrescine Concentrations

Hepatic putrescine levels were significantly lower in CCl_4-treated rats at 24 hours post PHx when compared to vehicle-treated rats ($p < 0.03$) regardless of treatment received - normal saline, low or high dose putrescine (figure 6). At 48 hours post PHx hepatic putrescine levels were similar between all groups of rats.

Putrescine Treatment and Hepatic Regeneration

All parameters of hepatic regeneration including restitution of liver mass, ODC activity and [^{3}H]-thymidine incorporation into DNA were similar in

Figure 3

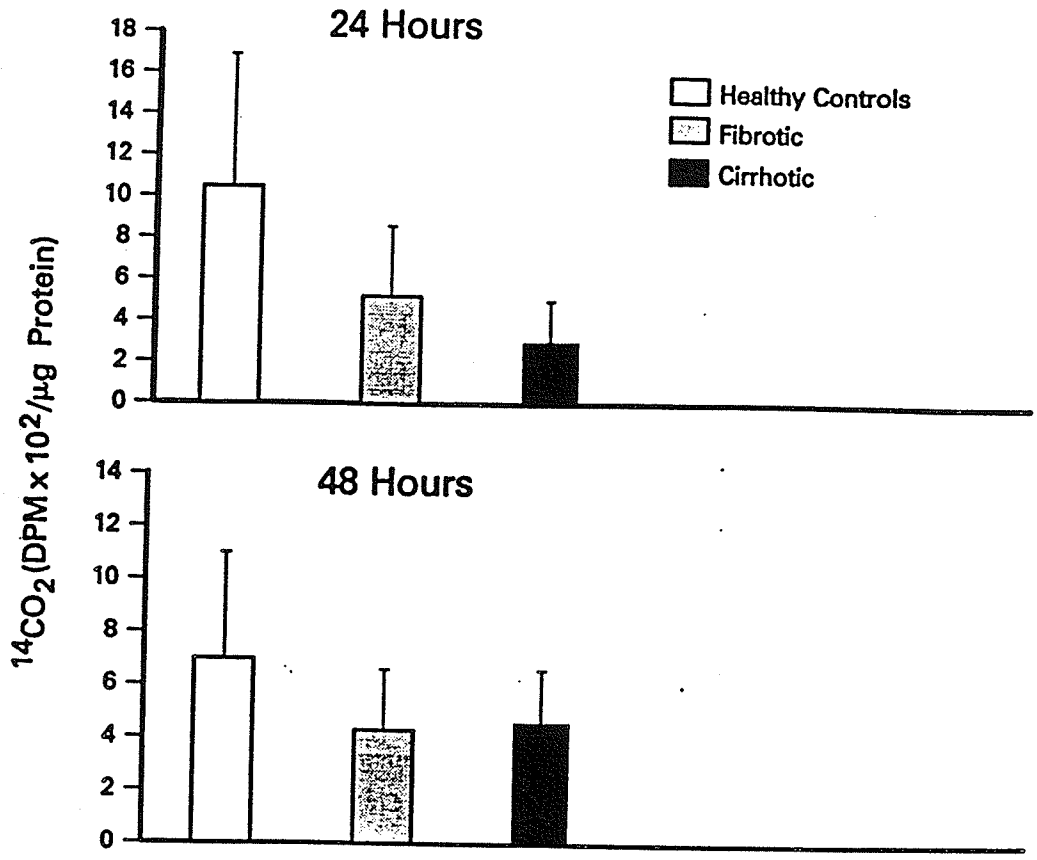

24 Hours
48 Hours
Healthy Controls
Fibrotic
Cirrhotic
$^{14}CO_2$(DPM x 10^2/µg Protein)
18
16
14
12
10
8
6
4
2
0
14
12
10
8
6
4
2
0

Figure 4

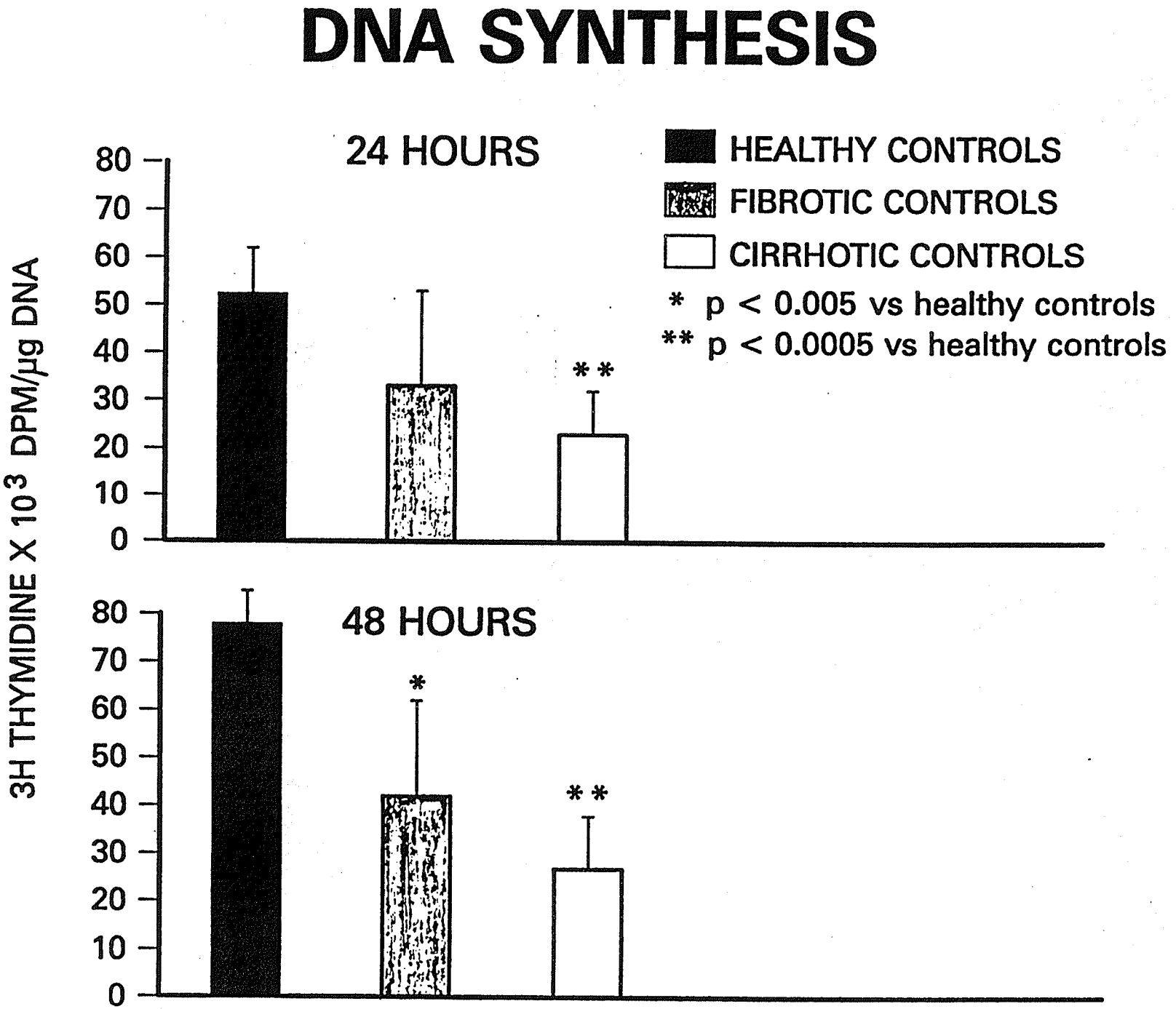

DNA SYNTHESIS
24 HOURS
48 HOURS
HEALTHY CONTROLS
FIBROTIC CONTROLS
CIRRHOTIC CONTROLS
* p < 0.005 vs healthy controls
** p < 0.0005 vs healthy controls
3H THYMIDINE X 10³ DPM/µg DNA
0
10
20
30
40
50
60
70
80
*
**

Figure 5

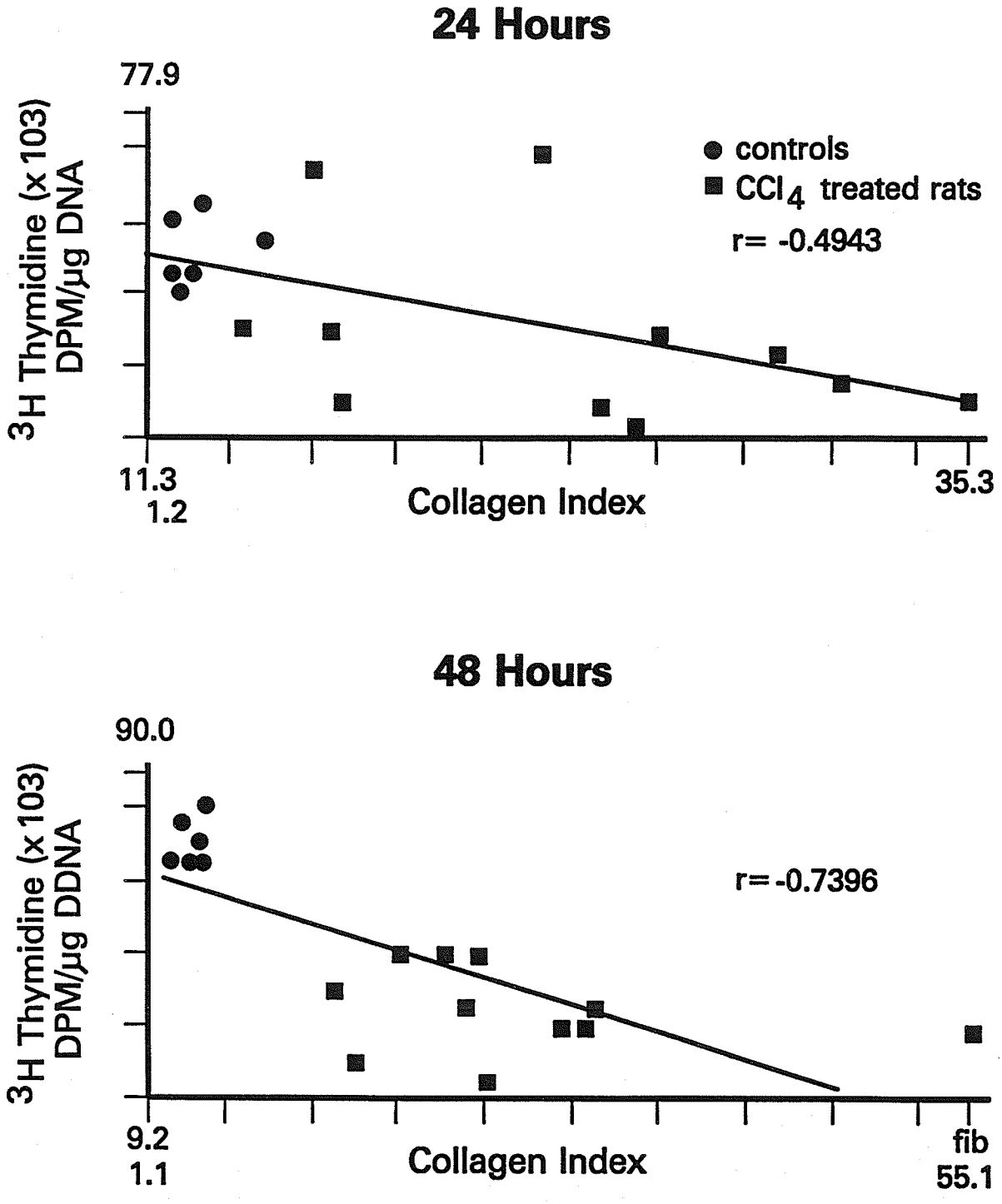
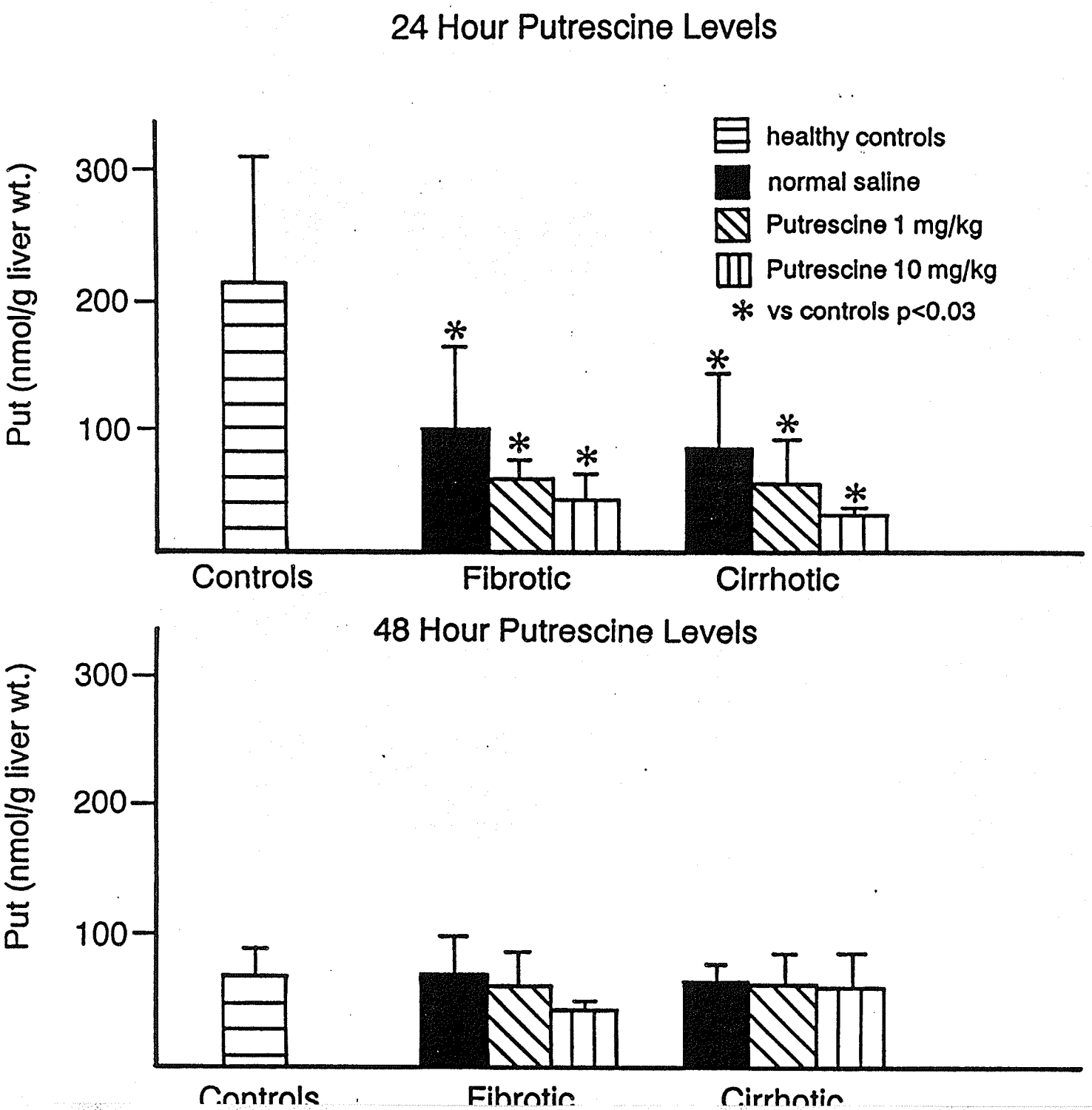

24 Hours
77.9
^{3}H Thymidine (x103)
DPM/µg DNA
controls
CCl_4 treated rats
r= -0.4943
11.3
1.2
Collagen Index
35.3
48 Hours
90.0
^{3}H Thymidine (x103)
DPM/µg DDNA
r= -0.7396
9.2
1.1
Collagen Index
fib
55.1

Figure 6


24 Hour Putrescine Levels
Put (nmol/g liver wt.)
300
200
100
healthy controls
normal saline
Putrescine 1 mg/kg
Putrescine 10 mg/kg
* vs controls p<0.03
Controls
Fibrotic
Cirrhotic
48 Hour Putrescine Levels
Put (nmol/g liver wt.)
300
200
100
Controls
Fibrotic
Cirrhotic

putrescine-treated rats (regardless of dose or schedule) when compared to their respective fibrotic and cirrhotic saline-treated controls (figures 7-9).

Figure 7

PERCENT RESTITUTION OF LIVER MASS

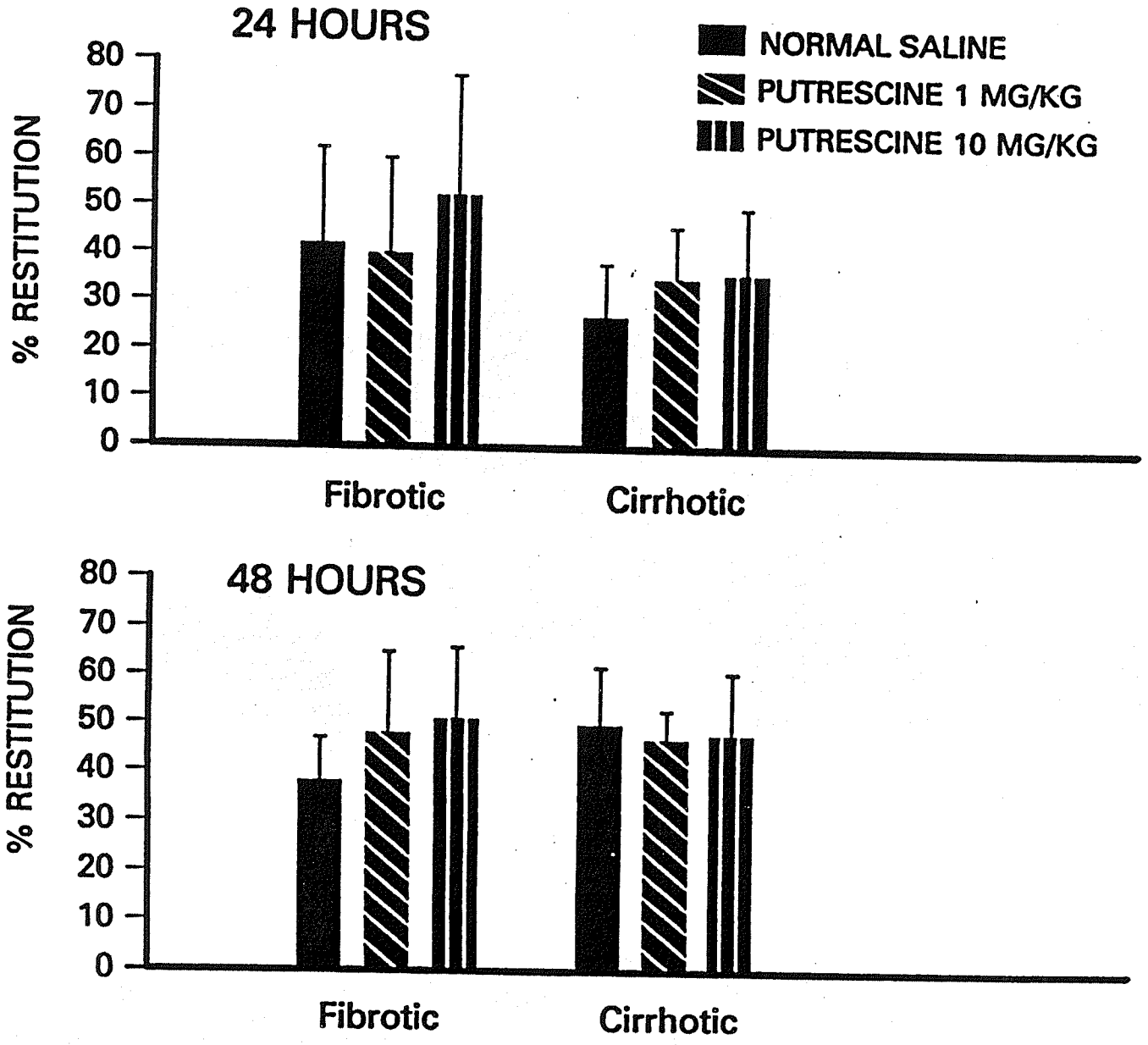


Figure 8

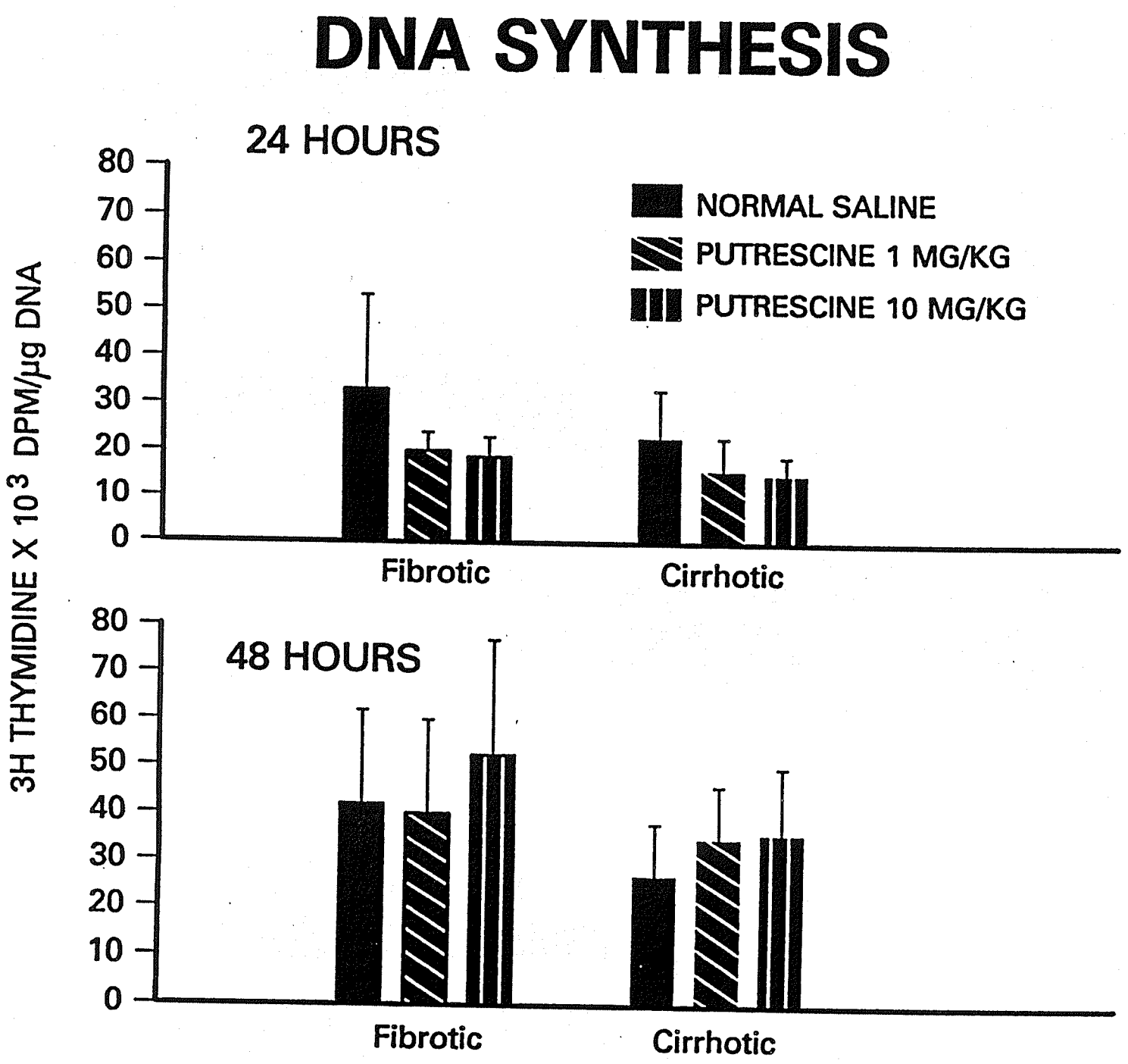
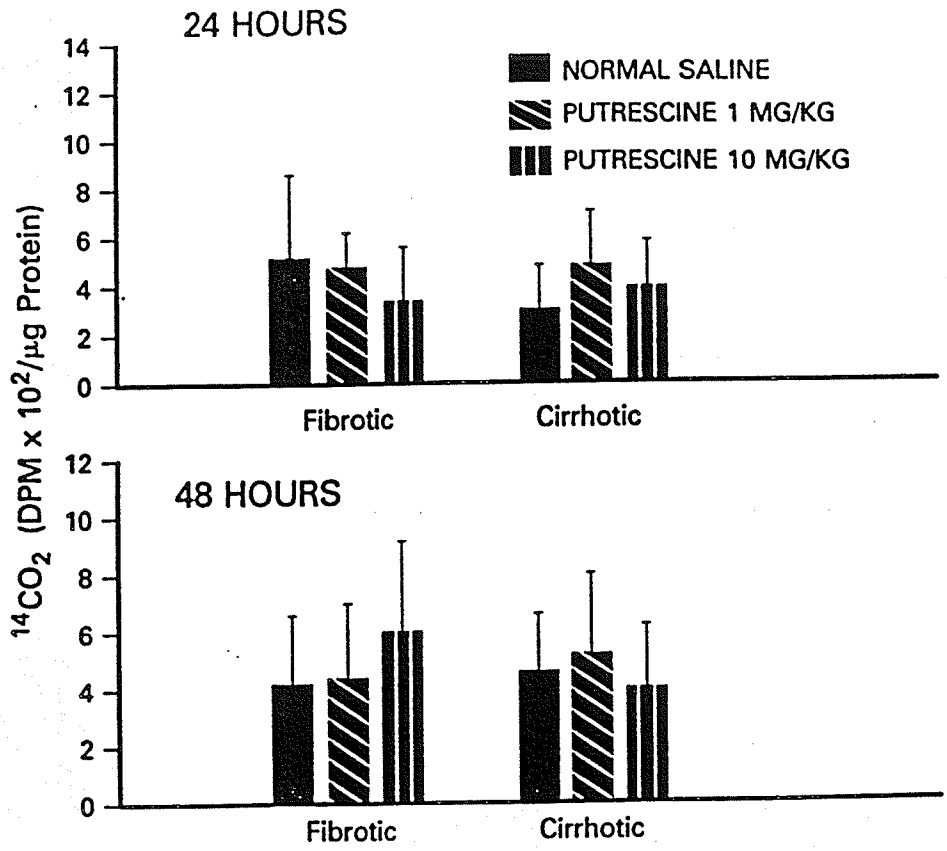

DNA SYNTHESIS
24 HOURS
NORMAL SALINE
PUTRESCINE 1 MG/KG
PUTRESCINE 10 MG/KG
3H THYMIDINE X 10³ DPM/µg DNA
80
70
60
50
40
30
20
10
0
Fibrotic
Cirrhotic
48 HOURS
80
70
60
50
40
30
20
10
0
Fibrotic
Cirrhotic

Figure 9


24 HOURS
NORMAL SALINE
PUTRESCINE 1 MG/KG
PUTRESCINE 10 MG/KG
$^{14}CO_2$ (DPM x 10^2/µg Protein)
0
2
4
6
8
10
12
14
Fibrotic
Cirrhotic
48 HOURS
0
2
4
6
8
10
12
Fibrotic
Cirrhotic

DISCUSSION

The results of the present work reflect the conflicting findings reported in the literature regarding the cirrhotic liver's ability to regenerate following partial hepatectomy. According to the restitution of liver mass and ODC data, hepatic regeneration is unaltered in cirrhotic rats when compared to healthy controls. Whereas according to the DNA synthesis data (as reflected by ^{3}H-thymidine incorporation into DNA), hepatic regeneration is significantly impaired in cirrhotic rats. Although the precise reason for these inconsistencies remains to be determined, a number of points regarding the parameters of regeneration employed in this and previous studies warrant further discussion.

Percentage restitution of liver mass is perhaps the most commonly employed parameter of hepatic regeneration, particularly in earlier studies. It is convenient, rapid and simple to perform. Unfortunately, it is a relatively inaccurate measure of hepatic regeneration as variations in stump size, inflammatory response and hepatic blood volume at the time of surgery can significantly affect the calculated liver mass (48). Moreover, the variable degrees of hypertrophy and atrophy that occur in cirrhotic livers tend to decrease the accuracy of liver weight determinations.

Numerous authors have reported the association between ODC activity and hepatic regeneration (49-52). However, because ODC has a strikingly short turnover time in mammalian cells (T 1/2 = 10-20 minutes) changes in ODC activity may occur very rapidly. As a result, ODC activity may not necessarily correlate with the results of other parameters of hepatic regeneration. For example, Diehl et al could not demonstrate a difference in ODC activity at 24 hours post partial hepatectomy in chronic ethanol fed rats compared to pair fed and ad libitum fed controls despite a significant decrease in ^{3}H-thymidine incorporation into DNA

in ethanol fed rats (50). Other authors have also emphasized that the time course of ODC activity is independent of DNA replication and cellular proliferation in the regenerating liver (51-53).

Despite the fact that the incorporation of ^{3}H-thymidine into hepatic DNA is generally considered a more useful and accurate reflection of regenerative activity (54), the results here indicate that this test must also be interpreted with caution. Specifically, in healthy young adult rats ^{3}H-thymidine incorporation into hepatic DNA normally peaks at 22-26 hours post partial hepatectomy whereas in the present study peak activity was delayed to 48 hours in control rats. This delay likely reflects the older age of the animals employed in the present study (10).

Assuming that the results of ^{3}H-thymidine incorporation into DNA were valid, the finding of decreased hepatic DNA synthesis at 48 hours in fibrotic rats and at 24 and 48 hours in cirrhotic rats support the results of Cameron and Karunaratue, who found no evidence of regenerative activity in CCl_4-treated rats with micronodular cirrhosis in response to PHx 72 hours earlier (16). However, it must be stressed that this may represent a delay in hepatic regeneration rather than an absolute loss of regenerative capacity.

The results of this study indicate that over a wide range of collagen contents (normal to cirrhosis) hepatic regenerative activity correlates with collagen content as determined by Van Giesen staining for collagen in the liver. However, the absence of a correlation between DNA synthesis rates and collagen contents in diseased livers (fibrosis and cirrhosis) suggests that other growth modulators play a more important role in influencing hepatic regenerative activity.

A further potential pitfall in using the collagen index to predict a regenerative response is that hepatic fibrosis is often not a uniform injury. For example, the CCl_4-induced injury model we employed affects smaller lobes more so than the larger lobes (11). This would have resulted in an underestimate of liver injury and fibrosis as the biopsy for automated image analysis was taken from the left lateral lobe, a large lobe of the liver. This inhomogeneity factor would be further compounded by the smaller sample sizes that are obtained with percutaneous and transjugular liver biopsy techniques.

Putrescine, a simple polyamine believed to play an important role in cell growth, has been reported to be essential for hepatic regenerative activity. In rats subjected to 70% PHx, pre-treatment with difluoromethylornithine (DFMO), a potent inhibitor of ODC and thereby polyamine synthesis, resulted in complete inhibition of hepatic regenerative activity which could only be restored by administration of exogenous putrescine (53). Recently Nishiguchi et al have reported that hepatic regeneration and survival is significantly enhanced in rats with chemical-induced hepatitis treated with exogenous putrescine (55). Diehl et al has provided similarly encouraging data in chronic ethanol fed rats (56). The reason why exogenous putrescine had no effect on hepatic regenerative activity in the cirrhotic rat is unclear. That the administration of exogenous putrescine did not enhance hepatic putrescine concentrations in the CCl_4-treated rats suggests either decreased delivery or impaired uptake by hepatocytes. One postulate is that intra- and extrahepatic shunting in cirrhotic rats results in decreased delivery of putrescine to the hepatocyte. However, it remains to be determined if the CCl_4 injured hepatocyte has been altered in its ability to transport putrescine into the cell.

SUMMARY

Hepatic regenerative activity is decreased in the early post operative period following partial hepatectomy in fibrotic and cirrhotic rats. The calculated hepatic collagen index does not predict hepatic regenerative response when the collagen content is increased. The decrease in hepatic regenerative activity is associated with decreased hepatic putrescine concentrations, however, exogenous putrescine does not restore hepatic regenerative activity to normal.

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